Author accepted MS: doi: http://dx.doi.org/10.1111/gfs.12163

Grass and Forage Science 2015 71, 139-152

Article title: Insensitivity of soil biological communities to phosphorus fertilisation in intensively-managed grassland systems

Authors names and affiliations:

Massey, P.A.[1,2,¶], Creamer, R.E.[1], Whelan, M.J [2,§] and Ritz, K[2,♯]*.

1. Teagasc, Environment, Soils and Land Use Research Department, Johnstown Castle, Ireland

2. School of Applied Sciences, Cranfield University, Cranfield MK43 0AL, UK

*Corresponding author (karl.ritz@nottingham.ac.uk)

Current addresses: [¶]Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, U.K.; [§]Department of Geography, Bennett Building, University of Leicester, Leicester, LE1 7RH, U.K.; [♯]Agricultural and Environmental Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD. karl.ritz@nottingham.ac.uk

Insensitivity of soil biological communities to phosphorus fertilisation in intensively-managed grassland systems

Massey, P.A.[1,2,¶], Creamer, R.E.[1], Whelan, M.J [2,§] and Ritz, K[2,♯*].[v]

1. Teagasc, Environment, Soils and Land Use Research Department, Johnstown Castle, Ireland

2. School of Applied Sciences, Cranfield University, Cranfield MK43 0AL, UK

Corresponding author (karl.ritz@nottingham.ac.uk)

Abstract

Efficient soil P cycling is essential for promoting optimal and sustainable grassland growth. The soil biological community is regarded as an important source of available P to the plant community. However, the effects of P fertilisation on the soil biota are unclear. This study aimed to investigate the effects of P fertilisation on plant and soil

[v] Current addresses: [¶]Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, U.K.; [§]Department of Geography, Bennett Building, University of Leicester, Leicester, LE1 7RH, U.K.; [♯]Agricultural and Environmental Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD. karl.ritz@nottingham.ac.uk

biological communities in two intensively managed grassland sites that had been receiving mineral-based P fertilisation over a 14-year period. Both pastures had been frequently cut and harvested for plant material and only received inorganic fertilisers since the establishment of the trial. Both sites were sampled four times from October 2009 to April 2011 and plant and a range of soil biological parameters were determined at each sampling period. The main findings of this study showed that soil chemical measures, such as labile inorganic P and total P concentrations, and plant yield and P contents responded as expected to P fertilisation. However, all soil biological parameters either showed no response or inconsistent responses to P fertilisation over the experimental period. This study indicates that intensive management regimes, for example intensive plant harvesting and fertiliser regimes, appear to over-ride the relationship between plant and soil biological communities with respect to their response to P fertilisation, and thus their productivity is apparently not predicated upon biotic activity.

Keywords

Phosphorus – Phosphorus fertilisation – Microbial biomass phosphorus – Plant phosphorus contents – Harvesting regime – Grassland systems

Introduction

The application of fertilisers to grassland systems is a common management practise that aims to increase growth and productivity of the vegetation. There are different fertiliser types that function by supplying specific nutrients to the soil. One such type includes inorganic P-based fertilisers that are commonly applied to adjust the P status of the grassland soil (Bunemann et al., 2011). When applied, such fertilisers produce a flush of bioavailable P within a short timeframe in the immediate vicinity of the area of application (Stewart, 1991; Stevenson and Cole, 1999). This flush of bioavailable P disrupts the equilibrium between P in the mobile phase (water-soluble bioavailable P) and the solid phase (bound P), which ultimately develops into the adsorption of water-soluble P to components of the soil fabric, rendering it unavailable for uptake by plants and soil microorganisms. This inability of the plant community to readily assimilate P that has become bound to the soil matrix results in either the impairment of grass growth or the over-compensation of applied fertiliser P by the farmer to ensure that enough bioavailable P is supplied to the vegetation to optimise such growth (Stewart, 1991).

Whilst soil chemical processes can affect P availability, the soil biological community is also regarded as an important factor governing the availability of P to plants (Brookes, 2001; Achat et al., 2010). For example, the turnover of microbial biomass has been shown to release labile pools of organic P which can be mineralised and ultimately utilised by grassland swards. Based on this manner of P cycling, the biomass can be viewed as a potential slow release pool of labile organic P into the soil that can be accessed by plants (Brookes, 2001). Nevertheless, the actual size and activity of the

80 microbial biomass is dependent on multiple soil factors, such as the presence of other
81 soil organisms, moisture content and soil type and the availability of other soil nutrients
82 (Gregorich et al., 1991; Thomsen et al., 2003; Demoling et al., 2007; Chapin III et al.,
83 2011). The turnover of the microbial biomass is critically linked to the activity of other
84 soil organisms. For example, soil nematode communities exhibit a range of trophic
85 interactions with other soil organisms. Some species feed exclusively on bacteria, some
86 on fungi and others are plant parasites (Ingham et al., 1985). By feeding on microbial
87 communities, nematodes have been shown to stimulate growth in the microbial
88 community and facilitate the release of soil nutrients, particularly N, from the microbial
89 biomass into the soil matrix (Ingham et al., 1985; Griffiths, 1994). By grazing on the
90 microbial community, the turnover rate of microbial biomass increases and released
91 nutrients aid in the synthesis of both microbial and plant biomass.

92 As stated above, soil type affects soil microbial communities. For example, increasing
93 clay content has been reported to increase the biomass nutrient pool size of the
94 microbial community due to a reduction in biomass turnover and an increase in soil
95 moisture content (Gregorich et al., 1991; Thomsen et al., 2003). A reduction in biomass
96 turnover occurs since increasing clay content facilitates the sorption of organic
97 compounds to the soil matrix, thereby increasing the resistance of the soil organic
98 matter pool to decomposition. Therefore, with increasing moisture content and a more
99 decomposition-resistant organic matter pool, the soil microbial community is less active
100 and biomass turnover occurs more slowly, which can result in the determination of
101 greater microbial biomass sizes with increasing clay contents. Like the effects of soil
102 type on biomass size, the size of the microbial biomass is also limited by the availability

of soil nutrients. One of the main nutrients known to be limiting microbial growth is C, which in turn limits assimilation of P (and other elements) (Demoling et al., 2007; Bunemann et al., 2011). With respect to C input into the soil, plants are recognised as being the primary source of C to the soil microbial community (Bardgett, 2005). Through rhizodeposition and plant senescence, plants supply different forms of C both directly to the soil surface via litter deposition (which may then be incorporated via the action of biota such as earthworms) and within the soil matrix via rhizodeposition. Such input of C stimulates the soil heterotrophic community and aids in soil nutrient cycling.

Whilst there are clear effects of soil biological communities on soil P cycling in the literature, the effects of P fertiliser application on soil biological communities are less apparent (Yeates, 1976; Ross et al., 1995; Sarathchandra et al., 2001; Gichangi et al., 2009). This ambiguity arises since varied results of P fertilisation have been reported on microbial and nematode communities. One common theme observed between these studies is linked to the joint application of both organic and inorganic fertiliser (Gichangi et al., 2009). The application of both forms has been shown to increase microbial biomass P concentrations above those observed from a sole application of inorganic fertiliser. However, when studies have investigated the sole application of inorganic P fertiliser, they either report inconsistent effects of P fertiliser on microbial biomass P over time or no effects at all (Ross et al., 1995; Sarathchandra et al., 2001). With respect to nematode communities, responses to P fertilisation appeared to be coupled to livestock grazing, arising via greater nutrient input into the soil as faecal material. This return of organic nutrients to the soil stimulates soil microbial growth, which in turn increases nematode numbers (Yeates, 1976).

With equivocal data from the literature regarding P fertilisation effects on soil biology under pastures, a study was conducted which utilised different soil types that had received long-term applications of inorganic P fertiliser (>14 years). The aim of this study was to investigate and to further clarify how plant and soil biological communities were affected by inorganic P fertiliser applications. We hypothesised that both plant and soil communities will respond to P fertilisation, and specifically that the application of P fertiliser will promote greater plant P yields and microbial biomass P concentrations.

Methods

Field description and sampling

All sampling was carried out on two long term grassland sites located in the dairy farm area at Johnstown Castle, Wexford, County Wexford, Ireland (latitude 52 ^{o}17 N, longitude 06^{o}30 W) (Daly, 2005). Site 1 is founded on a fine loamy soil (22% coarse sand, 27% fine sand, 29% silt and 22% clay) and Site 2 on a coarse loamy soil (29% coarse sand, 22% fine sand, 33% silt and 16% clay). Both of these sites received four different P application rates, *viz*. 0, 15, 30 and 45 kg P ha^{-1} y^{-1} of 16% single superphosphate (2 $CaSO_4$ + $Ca(H_2PO_4)_2$) (hereafter denoted P0, P15, P30 and P45, respectively). P was applied to each plot in February each year. There were four replicates of each treatment within a site and, therefore, 16 plots per site. Each plot had the dimension 10 x 2 m^2. Different P application rates were randomly positioned within a site according to a randomised block design. The plant species present in both grassland sites were *Lolium perenne* L., *Poa trivialis* L., *Agropyron repens* (L.) Beauv., *Trifolium repens* L. and various Agrostis spp. Aboveground plant material in both sites

148 was cut eight times per year to a height of 5 to 6 cm using a plot harvester. After
149 harvesting plant material in both sites, all plots received 40 kg N ha^{-1}, as calcium
150 ammonium nitrate (5 $Ca(NO_3)_2$ NH_4NO_3). Potassium was also applied as muriate of
151 potash (KCl) at a rate of 125 kg K ha^{-1} y^{-1} to compensate for potassium removal from
152 both systems. These trials were set up in February 1995 and thus had been established
153 for 14 years prior to the onset of this study.

154 Rainfall, air temperature and soil temperature were monitored over the experimental
155 period of this study. Rainfall was determined using a tipping bucket rain gauge. Air
156 temperature was measured using a dry platinum resistance thermometer. Soil
157 temperature was measured at a depth of 10 cm using a platinum resistance thermometer.

158 Soil samples were collected from both sites in October 2009, April 2010, October 2010
159 and April 2011. Five sample cores (diameter 6 cm, depth 10 cm) were collected from
160 each individual plot in a W-best-of-fit sampling design to ensure representative non-
161 biased sampling and composited on a per-plot basis.. Samples were firstly broken by
162 hand, homogenised and 100 g fresh weight subsampled for nematode analysis.
163 Remaining soil was coned, quartered and subsampled for 4 mm and 2 mm sieving to
164 remove the majority of plant and stone material. Soil sieved to 4 mm was subsampled
165 for phospholipid fatty acid analysis (PLFA) analysis and the soil that was sieved to 2
166 mm was subsampled for microbial biomass C, N and P. During the course of these
167 experiments, all fresh soil samples for microbial biomass analyses were stored at 4^oC.
168 Samples for PLFA analysis were stored at -80^oC using an ultra low temperature freezer
169 and freeze dried. Different sieve sizes were used in this experiment since the aim was
170 not to relate such properties but to examine how they were affected by P fertilisation.

171 By sieving to 4 mm for PLFA analysis, less disturbance of the soil matrix would occur
172 compared to 2 mm sieving (Petersen and Klug, 1994). Sieving to 2 mm was chosen for
173 all other parameters because this would enable for more comprehensive comparisons
174 with the literature, especially in the case of microbial biomass C, N and P
175 concentrations, since sieving to 2 mm is a more common practice for determining such
176 concentrations (Sarathchandra et al., 2001; Turner et al., 2001; Lui et al., 2010; Lui et
177 al., 2012). All remaining soil was dried at 40^{o}C, sieved to 2 mm and used to determine
178 soil pH, loss-on-ignition, Morgan extractable P and total P.

179 **Plant dry matter yield and phosphorus content**

180 All plant material harvested from each plot was dried at 70^{o}C for 72 h and weighed to
181 determine plant dry matter yield. Plant P contents were determined by digesting dried
182 plant subsamples using a Gerhardt Kjeldatherm block digester and measured using a
183 continuous flow peak height analyser (McCormack, 2002). Briefly, one No. 80
184 'Kjeltabs' tablet (Carl Stuart Limited, Republic of Ireland) was added to the dried plant
185 sample, 5 ml of 95% sulphuric acid (H_2SO_4) and 3 ml of hydrogen peroxide (H_2O_2)
186 were then added to the sample, heated at 150^{o}C for 1 h and then at 390^{o}C for 1.5 h using
187 the block digester. When digested, samples were diluted with deionised water to make
188 the sample volume up to 50 ml. Samples were then filtered and an aliquot was prepared
189 for P measurement.

190 Plant P content was determined based on orthophosphate concentrations produced
191 during digestion. Orthophosphate concentrations were determined by reacting

192 orthophosphate with a molybdovanadate reagent, which were then quantified
193 colourimetrically by reading absorbance at 420 nm (McCormack, 2002).

194 **Soil chemical methodologies**

195 Labile inorganic P was determined using the Morgan's extraction method (McCormack,
196 2002). Briefly, labile inorganic P was extracted from 3 g dry soil using 15 ml of a 0.62
197 M sodium hydroxide (NaOH) and 1.25 M acetic acid (CH_3COOH) solution adjusted to
198 pH 4.8 at a 1:5 (w/v) soil to solution ratio (Peech and English, 1944; McCormack,
199 2002). Soils were shaken for 30 minutes on gyratory shaker and filtered. P
200 concentrations were measured spectrophotometrically at 880 nm using the ammonium
201 molybdate-ascorbic acid method (Murphy and Riley, 1962). Total P concentrations
202 were determined in soil collected in April 2011 using the aqua regia digestion method
203 (ISO 11466:1995). Soil samples were ball milled to less than <0.1 mm and 2 g of this
204 milled material were weighed into digestion tubes. Both 16 ml of hydrochloric acid and
205 4 ml of nitric acid were added to each digestion tube, these tubes were then placed into a
206 digestion block. Samples were digested at 140^{o}C for 2 h, thereafter samples were
207 filtered using No. 2 Whatman filter paper and the filtered extract made up to a final
208 volume of 100 ml using 2 M nitric acid. Extracted P concentrations were measured
209 using inductively-coupled plasma optical-emission spectroscopy. Soil pH was
210 determined using a soil deionised water ratio of 1:2 (w/v) (McCormack, 2002). Organic
211 matter contents were determined by loss-on-ignition (Rowell, 1994)

212 **Soil biological methodologies**

213 Prior to the determination of microbial biomass C, N and P, all soil was incubated at
214 21^{o}C and field moisture content for 7 days. After this incubation period, microbial
215 biomass C and N concentrations were determined following a modified fumigation
216 extraction methodology (Brookes et al. 1985; Vance et al. 1987). Briefly, soil samples
217 were weighed out in duplicate; one sample for fumigation and another for baseline C
218 and N concentrations. Samples to be fumigated were placed into a vacuum desiccator
219 with ethanol-free chloroform and fumigated for 24 h. Following fumigation, samples
220 were extracted using 0.5 M potassium sulphate (K_2SO_4) (1:4 soil solution ratio) on a
221 side-to-side shaker for 30 min. The other subsamples were not fumigated but were
222 extracted using 0.5 M K_2SO_4 under the same conditions as the fumigated samples. After
223 extraction, C and N concentrations were measured using a Shimadzu TOC-V and TNM-
224 1 analyser (Shimadzu Corporation, Japan). Reported concentrations were corrected
225 using the conversion factors stated in the literature. Microbial biomass C and N
226 concentrations were estimated using the conversion factors 0.45 and 0.54, respectively
227 (Brookes et al. 1985; Vance et al. 1987).

228 Microbial biomass P concentrations were determined following the fumigation
229 extraction procedure (Brookes et al. 1982). Samples were weighed in triplicate; one for
230 fumigation, another for baseline P concentrations and a third to compensate for P
231 adsorption during the extraction process. The fumigation sample was fumigated using
232 ethanol-free chloroform in a vacuum desiccator for 24 h. Following fumigation, P was
233 extracted from fumigated and non-fumigated subsamples using a 0.5 M sodium
234 bicarbonate ($NaHCO_3$) solution adjusted to pH 8.5 at a 1:20 soil solution ratio. The third
235 sample, to account for P adsorption, was also extracted using 0.5 M $NaHCO_3$ but an

236 additional 1 ml of 25 μg P g^{-1} was added to the sample: 1 ml of deionised water was
237 added to fumigated and non-fumigated samples. P was applied as potassium dihydrogen
238 orthophosphate (KH_2PO_4). All samples were shaken for 30 min following the addition
239 of the extractant on a side-to-side shaker. Once extracted, P concentrations were
240 measured by UV spectroscopy at 880 nm following the ammonium molybdate-ascorbic
241 acid method (Murphy and Riley, 1962). Microbial biomass P was calculated using the
242 equation stated in Brookes et al. (1982).

243 PLFA analysis was determined using a modified procedure described by Frostegard et
244 al. (1991), which was based on the method described by Bligh and Dyer (1959). Lipids
245 were extracted from freeze dried samples using a mono-phase Bligh and Dyer
246 extractant. This reagent consisted of chloroform, methanol and 0.15 M citrate buffer
247 (1:2:0.8 v/v/v, respectively). With this extractant, samples were sonicated for 30 min
248 and then shaken for a further 30 min before being centrifuged. Using the organic top
249 layer of the extractant, phospholipids were separated from neutral lipids and glycolipids
250 in SPE cartridges (Silica Cartridge Sep Pak Vac 3 cc (500 mg), Waters Scientific, USA)
251 by solid phase extraction. Phospholipids were subjected to mild alkaline methanolysis
252 to form fatty acid methyl esters, which were then separated using gas chromatography
253 and detected using a flame ionisation detector set to 310^{o}C (Varian, USA). The column
254 used was a HP-5 (Agilent Technologies, USA) capillary column. A 26 Bacterial Acid
255 Methyl Ester mix was used as an external standard to identify commonly occurring
256 PLFA markers in the sample.

257 Nematodes were washed from 100 g fresh weight of soil using an Oostenbrink elutriator
258 (MEKU Erich Pollähne GmbH, Germany) (Seinhorst, 1962). This form of elutriation

passes a sample suspended in water through a series of sieves (180, 120, 95 and 52 µm apertures). Nematodes were separated from elutriated material using Baermann funnels over a 48 hr period. Following this period, 50 ml of sample was collected from the funnel. Samples were left for one day, after which 45 ml was removed. Constant checks were made to ensure that no nematodes were removed during this process. The remaining 5 ml of sample was mixed until nematodes were re-suspended and 2 ml transferred to a counting dish, to generate a representative measure of the total number of nematodes in the sample. The number of nematodes present in this 2 ml subsample was then counted using a light microscope. The abundance of nematodes (number g^{-1} dry soil) within the sample was then estimated based upon this representative subsample.

Earthworms were extracted using the Octet method (Schmidt, 2001) in April and October 2010. Vegetation was clipped to the soil surface and eight stainless steel electrodes were pushed into the soil (40 cm deep) at marked positions around a sampling ring (area 0.125 m^2). The stainless steel rods were joined to the sampling ring using the designated connector clips and the octet machine attached to a 105 AHR 12-V car battery. The machine was turned on and voltage increased from 200/250 V to 500/600 V at 5 min intervals for the first four steps and 10 min for the remaining two steps. Earthworms were collected from within the ring when they fully emerged. Live earthworms were then weighed, inclusive of gut content.

Statistics

Data were analysed using factorial repeated measures ANOVA via Statistica v.9 (Stats Soft, 2010). Site, P fertilisation rate and sampling occasion were designated as main effects, with sampling occasion as the repeated measure level. Total P data was analysed using factorial two-way ANOVA since data was only obtained in April 2011 and not a repeated measure. With respect to microbial community structure, all PLFA markers were normalised to mol% within each sample and analysed using principal component analysis (PCA) to produce principal components (PC) for the whole data-set. Resultant PCs were subsequently analysed using factorial repeated measures ANOVA to investigate treatment effects as stated above.

Results

Environmental conditions

With respect to the sampling occasions adopted for this experiment, the soil and air temperatures were similar at all four occasions (Fig. 1 (a)). The range of soil temperatures observed for these occasions were between 12.8 ± 0.17 and 10.3^{o}C ± 0.31 S.E. Air temperature during these occasions ranged from 12.1 ± 0.26 to 8.5^{o}C ± 0.36 S.E. The greatest difference between soil and air temperatures occurred in April 2010 (soil temperature; 10.3 ± 0.31, air temperature; 8.5^{o}C ± 0.36 S.E., whereas soil and air temperatures in October 2009, October 2010 and April 2011 were similar. Unlike soil and air temperatures, greater rainfall was observed in October 2009 and 2010 (5.2 ± 1.8 and 3 mm ± 0.8 S.E., respectively) compared to April 2010 and 2011 (0.9 mm ± 0.5 S.E. for both dates) (Fig. 1 (b)).

302

303 **Site and seasonal effects on soil chemical and biological parameters**

304 For most measured chemical and biological parameters, greater quantities and
305 concentrations were observed in the fine loamy soil compared to the coarse loamy soil.
306 No interactions between P fertilisation and Site were observed on both plant P and plant
307 dry matter contents ($p > 0.05$ for both parameters). When analysing plant P contents, a
308 significant site effect was apparent ($p < 0.01$), which showed that greater plant P
309 contents were manifest on the fine loamy compared to the coarse loamy soil (Table 1).
310 No site effects were observed on plant dry matter contents (Table 1).

311 No interaction between P fertilisation and Site was apparent on total soil P
312 concentrations ($p > 0.05$). However, a Site effect was observed ($p < 0.001$), which
313 showed greater total P concentrations in the fine loamy compared to the coarse loamy
314 soil (Fine loamy soil = 670; Coarse loamy soil = 517 mg kg^{-1} ± 14.2 pooled S.E.).
315 Morgan's P concentrations and organic matter contents were significantly affected by
316 Site whereas pH was not (Table 2). However, these site effects on Morgan's P and
317 organic matter differ between seasons, which were apparent by interactions with
318 sampling occasion (Table 2). The interactions for both of these parameters showed
319 greater concentrations and contents were measured in April 2010 and 2011 compared to
320 October 2009 and 2010 (Fig. 2 (a) and Fig. 3). Whilst no Site effect was apparent for
321 soil pH, a significant Site by Time interaction was observed (Table 2). This interaction
322 indicated a general decrease in pH in both sites, but the decrease in pH started on
323 different occasions for both sites. In the fine loamy soil, a consistent pH value was

observed until April 2010, thereafter pH decreased in October 2010 and decreased further in April 2011 (October 2009 = 6.5 ± 0.09; April 2010 = 6.6 ± 0.07; October 2010 = 6.3 ± 0.07; April 2011 = 6.01 ± 0.06 S.E.). In the coarse loamy soil, a consistent pH value was apparent until October 2010 after which pH decreased in April 2011 (October 2009 = 6.5 ± 0.09; April 2010 = 6.5 ± 0.07; October 2010 = 6.5 ± 0.07; April 2011 = 6.01 ± 0.06 S.E.).

Site effects were observed on microbial biomass C, N and P concentrations and on microbial community structure (Table 3). In general, microbial biomass C, N and P concentrations were greater in the fine loamy compared to coarse loamy soil. However, such site effects on all microbial parameters were confounded by interactions with sampling occasion. Microbial biomass C concentrations exhibited a complex third-order interaction between Site, P fertilisation treatment and Time (Table 3 and Fig. 4). Nevertheless, with respect to site temporal variation, there was greater variation in microbial biomass C concentrations in the coarse loamy soil across the experimental period compared to the fine loamy soil (Fig. 4). With respect to differences between sampling occasions, both sites showed a relatively large increase in microbial biomass C concentrations in April 2011 compared to all other sampling occasions. Microbial biomass P concentrations increased in the fine loamy soil over the experimental period (October 2009 = 47.4 ± 3.4; April 2010 = 70.7 ± 2.8; October 2010 = 66.9 ± 3.3; April 2011 = 90.4 $\mu g\ g^{-1}$ ± 2.9 S.E.), whereas in the coarse loamy soil, an increase occurred from October 2009 to April 2010, which was followed by consistent concentrations in October 2010 and April 2011 that were similar to April 2010 (October 2009 = 34.4 ± 3.4; April 2010 = 50.4 ± 2.8; October 2010 = 50.5 ± 3.3; April 2011 = 52.4 $\mu g\ g^{-1}$ ± 2.9

S.E.). With respect to microbial biomass N, greater concentrations were observed in the fine loamy soil in October 2009 and April 2011. In April 2010 and October 2010 similar concentrations were observed in both sites (Fine loamy soil: October 2009 = 184.7 ± 8.7; April 2010 = 100.6 ± 5.2; October 2010 = 152 ± 7.1; April 2011 = 212.3 μg g^{-1} ± 4 S.E. Coarse loamy soil: October 2009 = 123 ± 8.7; April 2010 = 93.8 ± 5.2, October 2010 = 155 ± 7.1; April 2011 = 151 μg g^{-1} ± 4 S.E.). Microbial phenotypic community structure showed marked significant variation over the experimental period. Such differences were apparent in PC1-3 (Table 3 and Fig. 5).

The period in which soil was sampled also had significant effects on nematode abundance and earthworm biomass (Table 3). With respect to nematode abundance, a significant Site by Time interaction was observed. In the fine loamy soil, nematode abundance was greater in April 2010 and 2011 compared to October 2009 and 2010 (October 2009 = 33.7 ± 2.25; April 2010 = 50.3 ± 2.88; October 2010 = 33.6 ± 2.68; April 2011 = 50.3 individuals g^{-1} dry soil ± 2.88 S.E.). In the coarse loamy soil, nematode abundance was significantly reduced in October 2009 compared to all other sampling dates (October 2009 = 24.6 ± 2.25; April 2010 = 40.2 ± 2.88; October 2010 = 42.7 ± 2.68; April 2011 = 40.2 individuals g^{-1} dry soil ± 2.88 S.E.). Regarding earthworm biomass, greater biomass was revealed in October 2010 (77.4 g m^2 ± 5.3 S.E) compared to April 2010 (55.3 g m^2 ± 4 S.E). A significant Site effect was observed on earthworm biomass ($p < 0.01$), which showed greater biomass in the fine loamy soil (77.3 g m^2 ± 4.9 S.E.) compared to the coarse loamy soil (55.4 g m^2 ± 4.9 S.E.).

Phosphorus fertiliser effects on soil chemical and biological parameters

The application of P fertiliser at different rates to both sites had significant effects on plant dry matter and P contents ($p < 0.001$ for both parameters). With respect to dry matter content, the application of P fertiliser up to a rate of 30 kg P ha^{-1} y^{-1} significantly increased dry matter production. The application rate at 45 kg P ha^{-1} y^{-1} produced the same dry matter content as the 30 kg P ha^{-1} y^{-1} treatment. Plant P contents increased with increasing P fertilisation rate (Table 1).

Total soil P concentrations were significantly affected by P fertilisation rate ($p < 0.001$). The P fertilisation effect revealed that total P concentrations generally increased with increasing P fertilisation rate (P0 = 504; P15 = 566; P30 = 590; P45 = 714 mg kg^{-1} ± 20 pooled S.E.). Specifically, *post hoc* analysis showed that total P concentrations in the P0 treatment were significantly lower than all other treatments, whereas concentrations in the P45 treatment were significantly greater than all other treatments. No significant difference between the P15 and P30 treatments was apparent. Morgan's P concentrations showed a significant P fertilisation x sampling occasion interaction (Table 2), manifest as a general increase in Morgan's P with increasing P fertilisation rate. However, greater variability in P concentration across all sampling occasions was observed in the P45 treatment compared to all other P fertilisation regimes (Fig. 2 (b)). No P fertilisation effect was observed on soil organic matter content (Table 2).

No P fertiliser effect or interaction was observed on microbial biomass P and N concentrations, nematode abundance or earthworm biomass (Table 3). With respect to microbial biomass C, no consistent effects of P fertiliser were observed across both sites

391 and sampling occasions, as apparent by a third-order interaction (Table 3 and Fig. 4).
392 This inconsistent effect revealed that different individual P treatments exhibited
393 significantly greater or lower microbial biomass C concentrations at particular sampling
394 occasions. Furthermore, microbial biomass C responses to P fertilisation across all
395 sampling occasions differed between the two sites.

396 No consistent P fertilisation effects were observed on microbial phenotypic community
397 structure. This inconsistent effect was manifest as a second-order interaction between P
398 fertilisation and Time for PC1, 2 and 3 (Table 3). These interactions revealed that
399 different individual P treatments were associated with community structures that were
400 significantly different from all other P treatments at each sampling occasion (Fig. 5 (a)
401 and (b)) No particular trends in microbial community structure are observed over time
402 between the different P treatments.

403 **Discussion**

404 **Seasonal and soil type effects on the soil biota in grassland systems**

405 All biological properties varied significantly between seasons in this study, with
406 microbial biomass C, N and P differing between all sampling periods, albeit with no
407 consistent trends between them. Many other studies have similarly reported temporal
408 variation in soil microbial properties (He et al., 1997; Krämer and Green, 2000; Chen et
409 al., 2003). Seasonal variations in microbial nutrient pools has been associated with
410 changes in soil moisture, soil temperature, root growth and activity (rhizodeposition)
411 and organic matter input through plant senescence (Chen et al., 2003). In the study
412 conducted by Chen et al. (2003), microbial biomass C and P were shown to vary

413 temporally, with greater variability observed in microbial biomass P than C. It appeared
414 that microbial biomass P was more sensitive to plant growth (thus plant P demand) and
415 soil moisture content compared to microbial biomass C. This result has also been
416 observed in another study by Tate et al. (1991). In particular, moisture deficit in the soil
417 may disrupt the diffusion of water-soluble P to the microbial community, thus affecting
418 microbial assimilation of P on a temporal scale (He et al., 1997). He et al. (1997)
419 explained that since a deficit in soil moisture content did not affect microbial biomass
420 C, but coincided with a decrease in microbial biomass P, then this may represent a loss
421 of P during microbial biomass P turnover and an inefficiency to then mobilise P from
422 the soil. This effect of moisture deficiency on microbial biomass P may also be
423 heightened by the activity of the plant community. The evapotranspiration induced by
424 the plant community would not only hinder the diffusion of water-soluble P to the
425 microbial community but could also be actively competing with the microbial
426 community for smaller concentrations of orthophosphate in the soil solution (He et al.,
427 1997). Therefore, the amount of P available to the microbial biomass may be severely
428 limited at specific times of the year and may explain why microbial biomass P
429 concentrations fluctuated during the experimental sampling period.

430 In this study, greater microbial biomass C, N and P was generally observed in the fine
431 loamy soil compared to the coarse loamy soil. This finding complements previous
432 studies focusing on textural effects upon C and N mineralisation and microbial biomass
433 C and N (Gregorich et al., 1991; Hassink, 1994). Furthermore, an increase in soil
434 organic matter with increasing clay content has also been reported (Gregorich et al.,
435 1991). This complements soil organic matter behaviour in this study, since the fine

loamy soil contained both greater organic matter contents and more silt and clay compared to the coarse loamy soil (4% and 6% more silt and clay, respectively). Soil organic matter has been shown to be greater with increasing clay content due to the greater sorption of organic compounds to the clay components of the soil matrix (Gregorich et al., 1991). Such sorption would increase the stability of organic matter and retard soil decomposition processes. With reduced organic matter decomposition, the microbial biomass becomes less active which results in a slower biomass turnover rate. Therefore, greater microbial biomass C, N and P pools were observed in the fine loamy soil as this reflects a slower turnover and less active microbial community compared to the microbial biomass in the coarse loamy soil (Gregorich et al., 1991; Hassink, 1994).

Plant and microbial responses to phosphorus fertilisation

This study highlights that the application of P fertiliser affected plant P yields but not soil microbial biomass P concentrations, despite an increase in labile inorganic P (Morgan's P) with increasing P fertilisation. Therefore, the hypothesis of this study was rejected since no increase in microbial biomass P was observed despite an increase in plant P yields. Other studies assessing the effect of P fertilisation on soil microbial biomass have generally found mixed responses in these circumstances. Some studies focusing on the long-term application of inorganic fertiliser P to grassland systems have found no effects on microbial biomass (Sarathchandra et al., 1993; Sarathchandra et al., 2001), whilst other studies have reported mixed responses to P fertilisation. Ross et al. (1995) presented results that showed inconsistent effects of rock phosphate on microbial biomass P concentrations in a New Zealand pasture which was analysed over a two year

459 period. This study conducted by Ross et al. (1995) found that microbial biomass P did
460 respond to P fertilisation on some sampling occasions, but not all. The application of
461 inorganic P fertiliser has also been shown to decrease microbial biomass C (Parfitt et
462 al., 2010). The explanation for this observation was attributed to a shift in microbial
463 community structure, since a more bacterial dominated community occurred in the
464 presence of a high fertility system that received inorganic P fertiliser. Consequently, a
465 more bacterial dominated community contributed to a lower microbial biomass C
466 concentration due to a more rapid turnover of such biomass due to grazing by micro-
467 fauna. In this present study, no consistent P fertilisation effects were observed on the
468 soil biota. Therefore, the absence of an effect in this instance does complement some
469 results from other grassland systems that have focused on the sole application of
470 inorganic P fertilisers.

471 In contrast, other studies have reported stimulatory effects of P fertilisation on the
472 microbial biomass P concentrations. In a maize-wheat crop rotation system, Lui et al.
473 (2010) reported that the application of inorganic P fertiliser combined with inorganic N
474 fertiliser increased microbial biomass P concentrations. It was, however, highlighted
475 that the greatest increase in microbial biomass occurred when inorganic and organic
476 fertiliser were applied together. In another study conducted by Parfitt et al. (2005), P
477 fertilisation effects were also observed on microbial biomass P concentrations in New
478 Zealand pastures that were grazed by sheep under both conventional and organic
479 fertiliser management. The P fertilisation effect was attributed to P limitation in the
480 control treatment, whilst no differences were observed between fertiliser management
481 types. One common theme that appears to be associated with the P fertiliser effects on

482 the soil biota is the importance of fertiliser type and management. When effects are
483 observed, they were seemingly stimulated particularly by the input of organic material.
484 In contrast, the fertiliser type and grassland management regime adopted in this present
485 study did not involve the input of organic matter into the system, since only inorganic
486 fertilisers were applied and no livestock were present. Furthermore, the intensive
487 harvesting regime adopted in this study, involving frequent and efficient removal of
488 plant biomass from the system, would have certainly curtailed plant-mediated inputs
489 into the soil. With respect to the importance of plant litter inputs to stimulate P demand
490 in the microbial community, a study by Liu et al. (2012) investigated how P additions to
491 forest systems under different management regimes affected microbial biomass P. Lui
492 et al. (2012) showed that the addition of P to a forest system that was not disturbed
493 increased both microbial biomass C and P, whereas P addition to a disturbed forest
494 system failed to invoke a response. It was concluded that C and N limitation in the
495 disturbed forest scenario may have limited P acquisition due to an absence of nutrient
496 accumulation. Therefore, we propose that one possible explanation for microbial
497 biomass P not responding to P fertilisation in the present study may be linked to the
498 management of these grassland sites, since such intensive harvesting may have
499 governed the availability of other soil nutrients. The removal of aboveground biomass
500 over a 16 year period would have constantly been removing C from the system and
501 manipulated plant behaviour (Nevens and Rehuel, 2003; Vinther, 2006; Ilmarinen and
502 Mikola, 2009; Ilmarinen et al., 2009). Cutting in this manner over such a long period of
503 time may have promoted similar rhizodeposition patterns across all P fertilisation
504 treatments since a greater investment in aboveground plant dry matter and P yields were

505 apparent with increasing P fertilisation. Therefore, by intensively cutting these
506 grassland sites, the response of soil biological communities to P fertilisation may be lost
507 as the plant community is the primary source of labile C to soil microbial communities
508 (Bardgett, 2005). This impact of similar C inputs may be manifest in this study through
509 the inconsistent effects of P fertilisation on microbial biomass C and microbial
510 community structure. In this context, the interaction between plant and microbial
511 communities in these grassland systems may have been driven by a similar input of
512 plant material across all P fertilisation treatments, which would have limited the
513 potential stimulatory effects of plant inputs on the microbial community in the presence
514 of P fertiliser.

515 Despite intensive C harvesting from these sites, microbial biomass C concentrations
516 were not low compared to other studies in grassland systems (Turner et al., 2001).
517 Turner et al. (2001) looked at microbial biomass C concentrations in a range of soil
518 types in 29 UK permanent grasslands. These concentrations ranged from 412 ± 19 to
519 3412 ± 21 $\mu g\ g^{-1}$. The largest concentrations observed were derived from soils with low
520 percentage sand contents and high clay contents. Larger concentrations of microbial
521 biomass C in the two grassland sites investigated in this study indicated that C may not
522 be the limiting nutrient. Further evidence for this was supported by soil loss-on-ignition
523 contents, which was similar to those observed in other Irish grasslands (Bourke et al.,
524 2008). Bourke et al. (2008) reported mean loss-on-ignition soil contents of 8.22%
525 (ranging from 6 - 10.9%) in sampled grassland sites at the Johnstown Castle Estate.
526 Thus, the accumulation of organic material in these two grassland sites was not reduced
527 by the cutting regime despite its intensity. This may support the conclusion that it was

528 the consistent inputs of similar quantities and qualities of C across all P fertilisation
529 regimes that was limiting microbial P acquisition in plots that received P fertilisation,
530 since no P fertilisation effect was observed on soil organic matter contents in this study.

531 **Conclusions**

532 From an agronomic perspective, both of these sites responded as expected to different
533 rates of P fertilisation. In particular, both sites showed that labile and total soil P
534 concentrations and plant yields and P contents increased with increasing P fertilisation.
535 However, despite consistent effects on soil P concentrations and the plant community,
536 no consistent P fertiliser effects were observed on soil biological communities. Such
537 differences between plant and soil biological communities may be linked to the
538 management of these grassland sites, since both sites were intensively managed via
539 exclusively inorganic mineral nutrient inputs and extensive removal of plant biomass.
540 This may have manipulated the interactions between plant and soil biological
541 communities, which could have potentially limited the acquisition of P by the soil
542 microbial community. One such management technique that may have greatly affected
543 the interaction both communities is the adopted harvesting regime. By adopting an
544 intense harvesting regime, the input of plant material into the soil may have been similar
545 across all P fertiliser treatments, resulting in different responses between plant and soil
546 biological communities. This study indicates that intensive management regimes appear
547 to decouple the relationship between plant and soil biological communities with respect
548 to their response to P fertilisation, and thus their productivity is apparently not
549 predicated upon soil biotic activity. The extent to which grasslands are then able to
550 carry any capacity to function when such fertiliser inputs cease is unclear, which is

551 critical considering the expense of P fertilisers to the agricultural community. Further
552 work is still required to truly define the interactive effects of multiple grassland
553 management techniques on the interactions between plant and soil biological
554 communities. By fully understanding these interactions, it may be possible to promote
555 greater P cycling in the soil, via the soil biota, which may open new insights into
556 reducing plant dependency on fertiliser applications.

557 **Acknowledgements**

558 This work was funded by the Teagasc Walsh Fellowship scheme. The authors wish to
559 thank the technical staff at the Teagasc Environment, Soils and Land-use Centre for
560 their support with laboratory analyses. We also thank two anonymous reviewers for
561 their insightful comments and suggestions for improvement of this paper.

562 **References**

563 ACHAT D.L., MOREL C., BAKKER M.R., AUGUSTO L., PELLERIN S., GALLET-
564 BUDYNEK A. and GONZALEZ M. (2010) Assessing turnover of microbial
565 biomass phosphorus: Combination of an isotopic dilution method with a mass
566 balance model. Soil Biology Biochemistry, **42**, 2231-2240.

567 BARDGETT R.D. (2005) *The Biology of Soil: A Community and Ecosystem Approach*.
568 Oxford, UK: Oxford University Press.

569 BARDGETT R.D. and MCALISTER E. (1999) The measurement of soil fungal:
570 bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate
571 meadow grasslands. Biology and Fertility of Soils, **29**, 282-290.

572 BARDGETT R.D. and SHINE A. (1999) Linkages between plant litter diversity, soil
573 microbial biomass and ecosystem function in temperate grasslands. Soil Biology
574 and Biochemistry, **31**, 317-321.

575 BOURKE D., DOWDING P., TUNNEY H., O'BRIEN J. and JEFFREY D.W. (2008)
576 The organic phosphorus composition of an irish grassland soil. Biology and
577 Environment: Proceedings of the Royal Irish Academy, **108B**, 17-28.

578 BLIGH E.G. and DYER W.J. (1959) A rapid method of total lipid extraction and
579 purification. Canadian Journal of Biochemistry and Physiology, **37**, 911-917.

580 BROOKES P.C. (2001) The soil microbial biomass: concept, measurement and
581 applications in soil ecosystem research. Microbes and Environments, **16**, 131-140.

582 BROOKES P.C., POWLSON D.S. and JENKINSON D.S. (1982) Measurement of
583 microbial biomass phosphorus in soil. Soil Biology and Biochemistry, **14**, 319-329.

584 BROOKES P.C., LANDMAN A., PRUDEN G. and JENKINSON D.S. (1985)
585 Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction
586 method to measure microbial biomass nitrogen in soil. Soil Biology and
587 Biochemistry, **17**, 837-842.

588 BUNEMANN E.K., OBERSON A. and FROSSARD E. (2011) *Phosphorus in Action:*
589 *Biological Processes in Soil Phosphorus Cycling.* Berlin, Germany: Springer-
590 Verlag.

591 CHAPIN III F.S., MATSON P.A. and MOONEY H.A. (2011) *Principles of Terrestrial*
592 *Ecosystem Ecology*, 2nd edn., USA: Springer Science and Business Media Inc.

593 CHEN C., CONDRON L., SINAJ S., DAVIS M., SHERLOCK R. and FROSSARD E.
594 (2003) Effects of plant species on phosphorus availability in a range of grassland
595 soils. Plant and Soil, **256**, 115-130.

596 DEMOLING F., FIGUEROA D. and BAATH E. (2007) Comparison of factors limiting
597 bacterial growth in different soils. Soil Biology and Biochemistry, **39**, 2485-2495.

598 FROSTEGARD. A., TUNLID A. and BAATH E. (1991) Microbial biomass measured
599 as total lipid phosphate in soils of different organic content. Journal of
600 Microbiological Methods, **14**, 151-163.

601 GICHANGI E.M., MNKENI P.N.S. and BROOKES P.C. (2009) Effects of goat manure
602 and inorganic phosphate addition on soil inorganic and microbial biomass
603 phosphorus fractions under laboratory incubation conditions. Soil Science and
604 Plant Nutrition, **55**, 764-771.

605 GREGORICH E.G., VORONEY R.P. and KACHANOSKI R.G. (1991) Turnover of
606 carbon through the microbial biomass in soils with different texture. Soil Biology
607 and Biochemistry, **23**, 799-805.

608 GRIFFITHS B.S. (1994) Microbial-feeding nematodes and protozoa in soil: Their
609 effects on microbial activity and nitrogen mineralization in decomposition hotspots
610 and the rhizosphere. Plant and Soil, **164**, 25-33.

611 HASSINK J. (1994) Effect of soil texture on the size of the microbial biomass and on
612 the amount of C and N mineralized per unit of microbial biomass in Dutch
613 grassland soils. Soil Biology and Biochemistry, **26**, 1573-1581.

614 HE Z.L., WU J., O'DONNELL A.G. and SYERS J.K. (1997) Seasonal responses in
615 microbial biomass carbon, phosphorus and sulphur in soils under pasture. Biology
616 and Fertility of Soils, **24**, 421-428.

617 ILMARINEN K. and MIKOLA J. (2009) Soil feedback does not explain mowing
618 effects on vegetation structure in a semi-natural grassland. Acta Oecologica, **35**,
619 838-848.

620 ILMARINEN K., MIKOLA J., NISSINEN K. and VESTBERG M. (2009) Role of soil
621 organisms in the maintenance of species-rich seminatural grasslands through
622 mowing. Restoration Ecology, **17**, 78-88.

623 INGHAM R.E., TROFYMOW J.A., INGHAM E.R. and COLEMAN D.C. (1985)
624 Interactions of bacteria, fungi, and their nematode grazers-effects on nutrient
625 cycling and plant-growth. Ecological Monographs, **55**, 119-140.

626 ISO 11466:1995 – Soil Quality part 3: Chemcial Methods section 3.9 – Extraction of
627 trace elements soluble in aqua regia.

628 KRÄMER S. and GREEN D.M. (2000) Acid and alkaline phosphatase dynamics and
629 their relationship to soil microclimate in a semiarid woodland. Soil Biology and
630 Biochemistry, **32**, 179-188.

631 LIU E., YAN C., MEI X., HE W., BING S.H., DING L., LIU Q., LIU S. and FAN T.
632 (2010) Long-term effect of chemical fertilizer, straw, and manure on soil chemical
633 and biological properties in northwest China. Geoderma, **158**, 173-180.

634 LIU L., GUNDERSEN P., ZHANG T. and MO J. (2012) Effects of phosphorus addition
635 on soil microbial biomass and community composition in three forest types in
636 tropical China. Soil Biology and Biochemistry, **44**, 31-38.

637 MCCORMACK S. (2002) *Analysis of Agricultural Materials: Methods used at*
638 *Johnstown Castle Research Centre*, 2 edn. Teagasc Environment, Soils and Land-
639 use Research Centre, Wexford, Republic of Ireland: Teagasc: Agricultural and
640 Food Development Authority.

641 MURPHY J. and RILEY J.P. (1962) A modified single-solution method for the
642 determination of phosphate in natural waters. Analytica Chimica Acta, **27**, 31-36.

643 NEVENS F. and REHUEL D. (2003) Effects of cutting or grazing grass swards on
644 herbage yield, nitrogen uptake and residual soil nitrate at different levels of N
645 fertilisation. Grass and Forage Science, **58**, 431-449.

646 PARFITT R.L., YEATES G.W., ROSS D.J., SCHON N.L., MACKAY A.D. and
647 WARDLE D.A. (2010) Effect of fertilizer, herbicide and grazing management of
648 pastures on plant and soil communities. Applied Soil Ecology, **45**, 175-186.

649 PEECH M. and ENGLISH L. (1944) Rapid microbiological soil tests. Soil Science, **57**,
650 167-195.

651 PETERSEN S.O. and KLUG M.J. (1994) Effects of sieving, storage, and incubation-
652 temperature on the phospholipid fatty-acid profile of a soil microbial community.
653 Applied Environmental Microbiology, **60**, 2421-2430.

654 ROSS D.J., SPEIR T.W., KETTLES H.A. and MACKAY A.D. (1995) Soil microbial
655 biomass, C and N mineralization and enzyme activities in a hill pasture: Influence
656 of season and slow-release P and S fertilizer. Soil Biology and Biochemistry, **27**,
657 1431-1443.

658 SARATHCHANDRA S.U., GHANI A., YEATES G.W., BURCH G. and COX N.R.
659 (2001) Effect of nitrogen and phosphate fertilisers on microbial and nematode
660 diversity in pasture soils. Soil Biology and Biochemistry, **33**, 953-964.

661 SARATHCHANDRA S.U., LEE A., PERROTT K.W., RAJAN S.S.S., OLIVER
662 E.H.A. and GRAVETT I.M. (1993) Effects of phosphate fertilizer applications on
663 microorganisms in pastoral soil. Australian Journal of Soil Research, **31**, 299-309.

664 SCHMIDT O. (2001) Appraisal of the electrical octet method for estimating earthworm
665 populations in arable land. Annuals of Applied Biology, **138**, 231-241.

666 SEINHORST J.W. (1962) Modifications of the elutriation method for extracting
667 nematodes from soil. Nematologica, **8**, 117-128.

668 Statistica v9 (2010), USA: Stats Soft Inc.

669 STEVENSON F.J. and COLE M.A. (1999) The phosphorus cycle. In: Stevenson F.J.
670 and Cole M.A. (eds) *Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur,*
671 *Micronutrients*, 2 edn, pp.279-329. USA: John Wiley & Sons Inc.

672 STEWART B.A. (1991) *Advances in soil science*. USA: Springer-Verlag New York
673 Inc.

674 TATE K.R., SPEIR T.W., ROSS D.J., PARFITT R.L., WHALE K.N. and COWLING
675 J.C. (1991) Temporal variations in some plant and soil P pools in two pasture soils
676 of widely different P fertility status. Plant and Soil, **132**, 219-232.

677 THOMSEN I.K., SCHJØNNING P., OLESEN J.E. and CHRISTENSEN B.T. (2003)
678 Carbon and nitrogen turnover in structurally intact soils of different texture. Soil
679 Biology and Biochemistry, **35**, 765-774.

680 TURNER, B.L., BRISTOW, A.W. and HAYGARTH P.M. (2001) Rapid estimation of
681 microbial biomass in grassland soils by ultra-violet absorbance. Soil Biology and
682 Biochemistry, **33**, 913-919.

683 VANCE E.D., BROOKES P.C. and JENKINSON D.S. (1987) An extraction method
684 for measuring soil microbial biomass carbon. Soil Biology and Biochemistry, **19**,
685 703-707.

686 VINTHER F.P. (2006) Effects of cutting frequency on plant production, N-uptake and
687 N2 fixation in above- and below-ground plant biomass of perennial ryegrass-white
688 clover swards. Grass and Forage Science, **61**, 154-163.

689 YEATES G.W. (1976) Effect of fertiliser treatment and stocking rate on pasture
690 nematode populations in a yellow-grey earth. New Zealand Journal of Agricultural
691 Research, **19**, 405-408.

692

693 **Figure captions**

694 **Fig. 1** Environmental conditions in the Johnstown Castle estate from October 2009 to
695 April 2011, (a) denotes air and soil temperature and (b) denotes rainfall. Error bars
696 denote standard error.

697 **Fig. 2** Mean Morgan's P concentrations expressed by (a) the interaction between sites
698 and sampling periods and (b) the interaction between P fertilisation rates and sampling
699 periods. Same letters denote homogeneous means ($p<0.05$) using Fisher least significant
700 difference test. Error bars denote standard error.

701 **Fig. 3** Interactions between sites and sampling periods with respect to soil organic
702 matter content. Same letters denote homogeneous means ($p<0.05$) using Fisher least
703 significant difference test. Error bars denote standard error.

704 **Fig. 4** Interactions between site, sampling period and P fertilisation (Abbreviated as P:
705 numerals denote application rate kg^{-1} ha^{-1} y^{-1}) on microbial biomass C concentrations
706 (Third-order interaction significance $p = 0.028$). There are 15 homogenous mean
707 groups associated with this interaction, hence letters denoting these groups have been
708 omitted for clarity. Error bars denote pooled standard error.

709 **Fig. 5** Differences in phenotypic microbial community structure in the presence of four
710 different P fertilisation regimes (Abbreviated as P: numerals denote application rate kg^{-1}
711 ha^{-1} y^{-1}) at four sampling periods as expressed by (a) PC1 and PC2 and (b) PC2 and
712 PC3. Percentage variation accounted for by respective PCs shown in parenthesis. The
713 bounding ellipses drawn around each sampling time are to assist visualisation, and have
714 no formal statistical derivation.

715

716 **Table 1** Mean total plant dry matter yields and total plant P contents over the course of this study in the
717 presence of four P fertiliser regimes and across two grassland sites. Site 1 is a fine loamy soil and Site 2 is
718 a coarse loamy soil. Same Letters denote homogeneous means ($p<0.05$) using Fisher least significant
719 difference test. Standard error is represented as error mean squares (EMS).

	(kg m^2)	(g m^2)
P fertilisation rate (kg P ha^{-1} y^{-1})	**Total dry matter**	**Total plant P**
0	1.38 a	1.87 a
15	1.67 b	2.93 b
30	1.79 c	3.86 c
45	1.79 c	4.42 d
EMS	0.01	0.07
Site		
1 (Fine loam)	1.67 a	3.44 a
2 (Sandy loam)	1.64 a	3.10 b
EMS	0.01	0.07

720

721

722 **Table 2** ANOVA table showing the effects and interactions of Site, P fertilisation rate and sampling
723 occasion (Time) on general soil parameters.

	Morgan's P (µg g^{-1})	**pH**	**Loss-On-Ignition (%)**
Site	***	-	***
P treatment	***	-	-
Time	***	***	***
Site x Time	**	*	***
P treatment x Time	***	-	-
Site x P treatment	-	-	-
Site x P treatment x Time	*	-	-

724

725 Significance terms denote *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; - $p > 0.05$, respectively.

726

727

728 **Table 3** ANOVA table showing the effects and interactions of Site, P fertilisation rate and sampling
729 occasion (Time) on measured soil biological parameters.

	Microbial biomass (µg g^{-1})			Microbial community structure			Soil faunal	
	C	**N**	**P**	**PC1 (38%)**	**PC2 (16%)**	**PC3 (11%)**	**Earthworm biomass (g m^{-2})**	**Nematode abundance (number g^{-1} dry weight)**
Site	***	***	***	-	***	***	**	-
P treatment	**	-	-	*	-	-	-	-
Time	***	***	***	***	***	***	**	***
Site x Time	***	***	***	-	-	-	-	***
P treatment x Time	**	-	-	*	**	*	-	-
Site x P treatment	-	-	-	-	-	-	-	-
Site x P treatment x Time	***	-	-	-	-	-	-	-

730

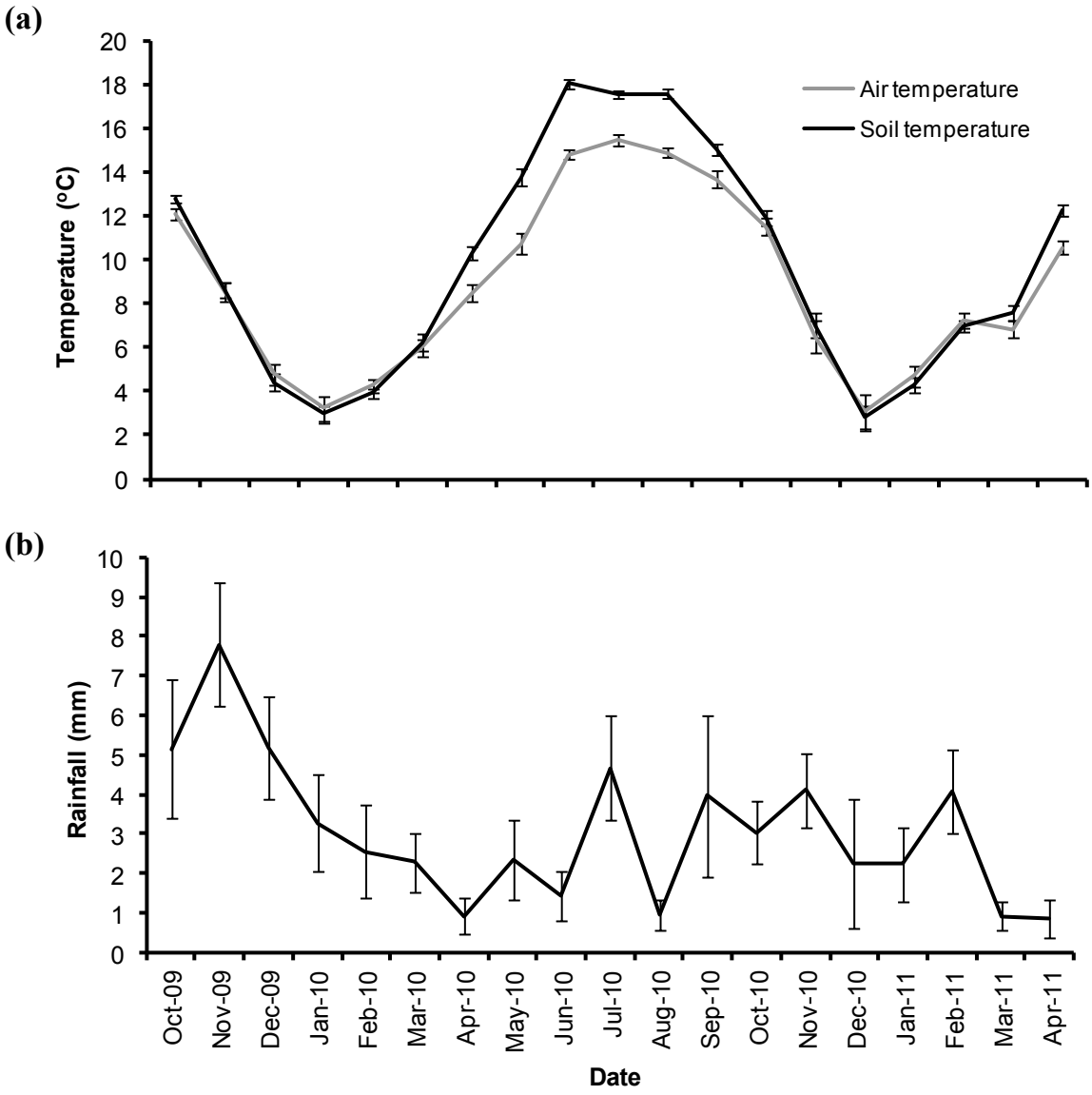
731 Significance terms denote *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; - $p > 0.05$, respectively.

732

733

734 Figure 1

735



736

737

738

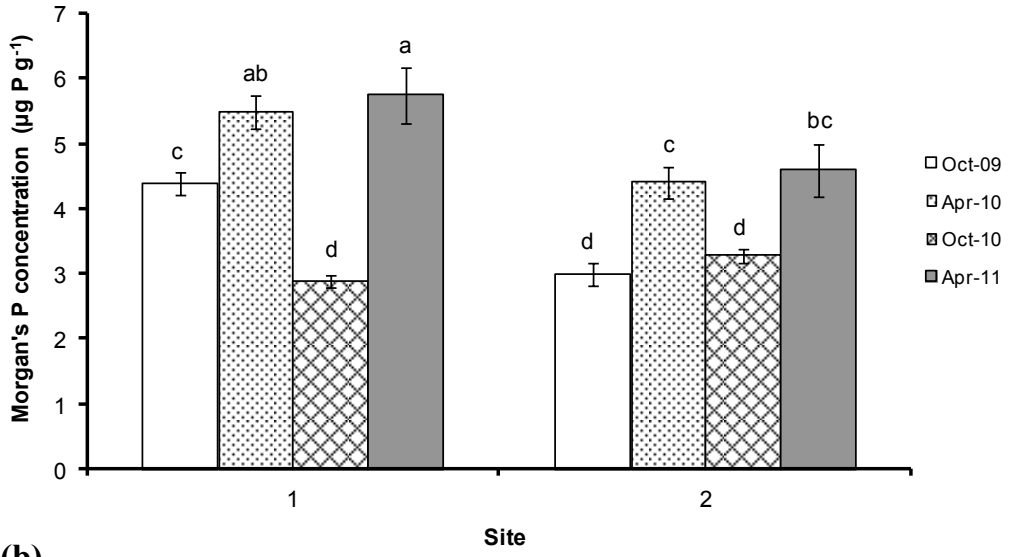
739

740

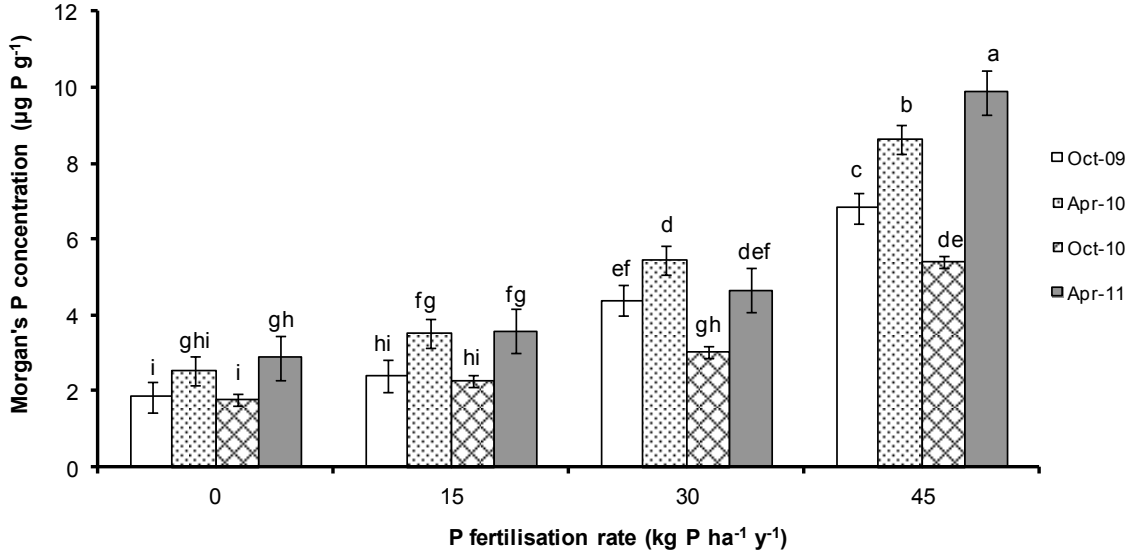
741 Figure 2

742 **(a)**

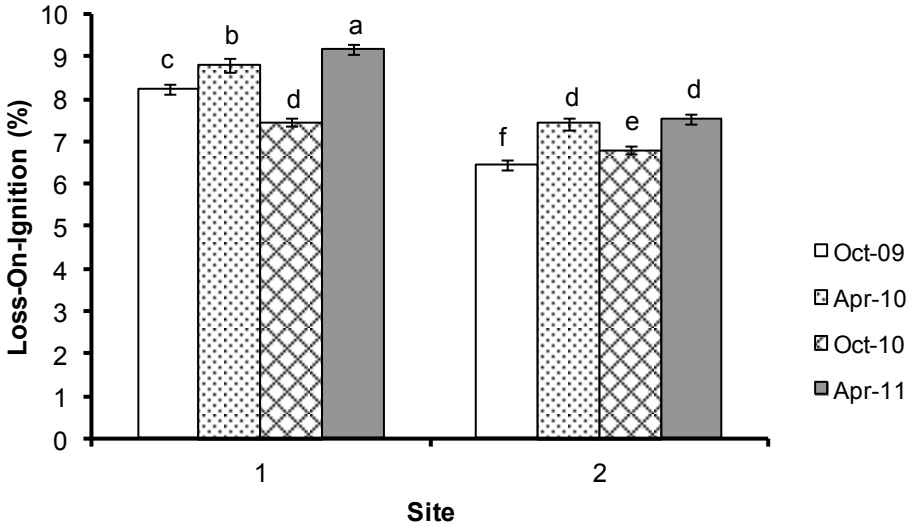
743



(b)

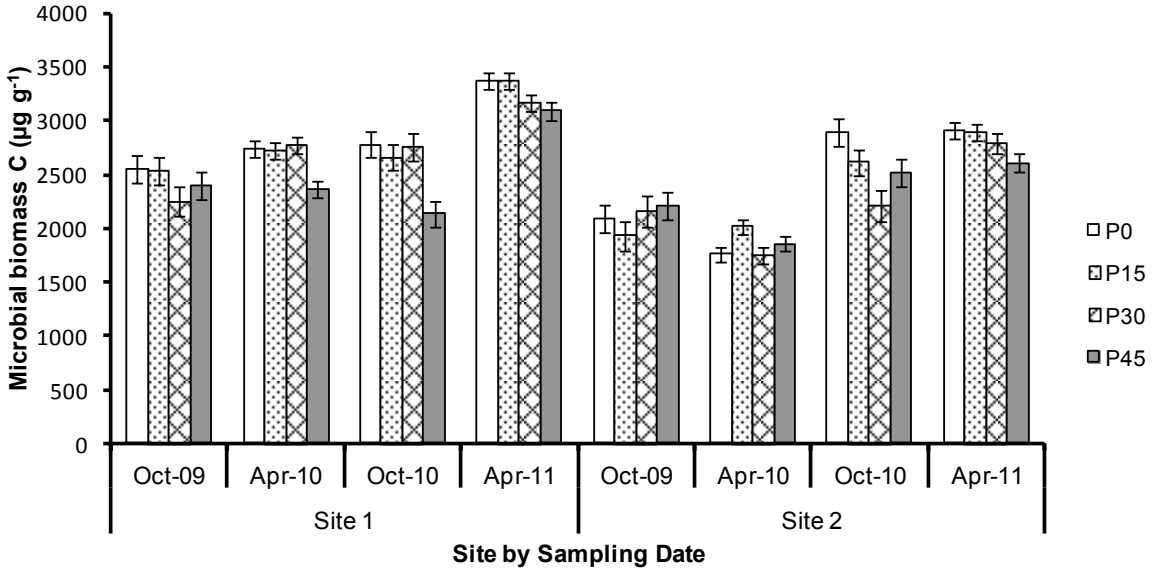


744 Figure 3



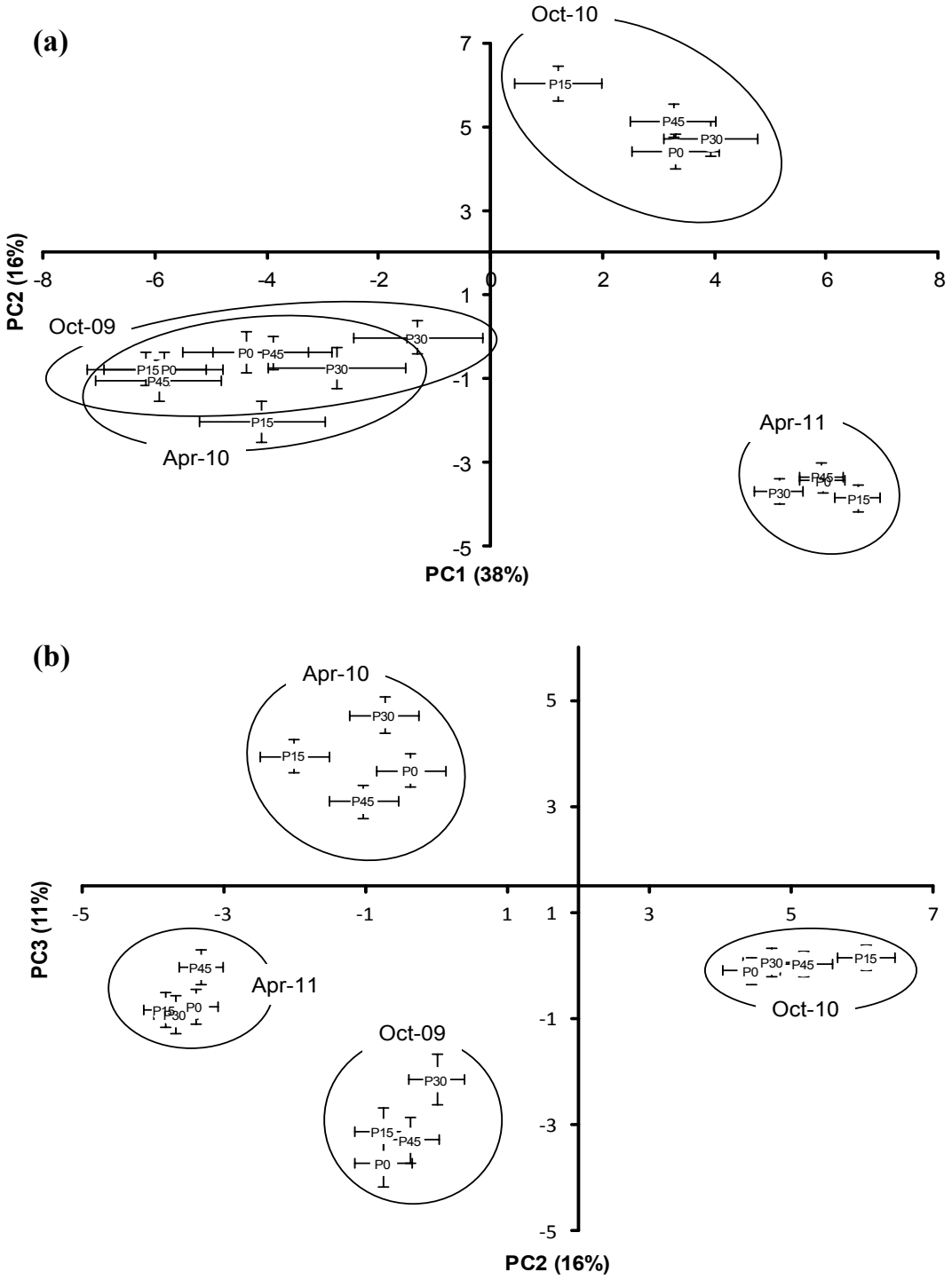
745

746 Figure 4



747

748 Figure 5



749